Discovery of a Potent, Orally Active, Nonsteroidal Androgen Receptor Agonist: 4-Ethyl-1,2,3,4-tetrahydro-6-(trifluoromethyl)-8-pyridono[5,6-*g*]-quinoline (LG121071)

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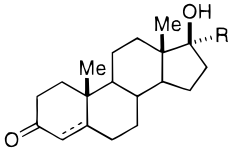
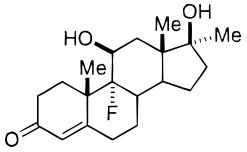
Introduction. Deficiencies in circulating levels of the androgens testosterone (T, **1a**) and dihydrotestosterone (DHT, **1b**) in hypogonadal men can be compensated for by administration of exogenous androgens,[1,2] which have proven efficacious in hormone replacement therapy, abrogating age-related deterioration of muscle and bone,[3] and regulating plasma lipids.[4] Cancer cachexia,[5] male contraception,[6] and performance enhancement[7] have also been investigated as clinical targets of androgen therapy.[8]



1a, R = H, $\Delta^{4,5}$
b, R = H
c, R = Me, $\Delta^{4,5}$



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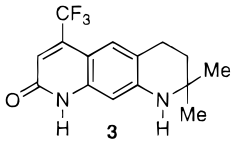

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The beneficial effects of administered steroidal androgens are often overshadowed by their rapid metabolic conversion to DHT by 5α-reductase and to estrogens by aromatase, resulting in side effects. Circumventing this metabolism through alternate routes of administration, such as intramuscular injection or transdermal patch applied to the scrotal skin,[9] and attempts to improve oral half-life of T using long-chain alkyl esters as prodrugs have met with limited success.[10] Alkylation of androgens at C-17, as in methyltestosterone (**1c**) and fluoxymesterone (**2**), has been observed to slow hepatic metabolism, allowing oral administration, but subsequent liver toxicity limits their use for chronic administration.

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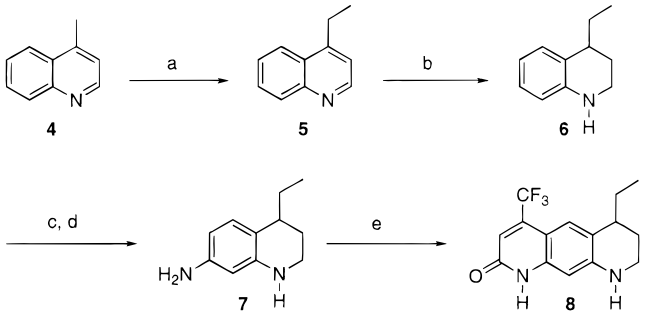
Scheme 1[a]



[a] (a) LDA, MeI, THF, −78 °C, 98%; (b) $NiCl_2 \cdot 6H_2O$, $NaBH_4$, MeOH, 0 °C−rt, 96%; (c) HNO_3, H_2SO_4, −10 °C, 10 min, 91%; (d) H_2, 10% Pd/C, EtOH/EtOAc, rt, 16 h, 94%; (e) ethyl 4,4,4-trifluoroacetoacetate, $ZnCl_2$, EtOH, reflux, 8 h, 82%.

Efforts to identify more receptor- and tissue-selective compounds which might avoid steroid-related side effects and toxicities have shifted focus away from steroid structural templates. Though representatives of several structural classes have been developed or are currently undergoing late-stage preclinical development as human androgen receptor (hAR) antagonists,[10] efforts to discover and develop nonsteroidal AR agonists have been few.[11] To date, no known nonsteroidal AR agonists have been reported to exhibit activity in vivo. In the course of our investigations into the structure−activity relationships of dihydroquinoline-based AR antagonists such as **3**,[12] we had the opportunity to examine the effect of removal of 2,2-dialkyl substitution. It had previously been noted that substitution at this position played a critical role in driving the receptor into a transcriptionally inactive conformation and that lack of geminal substitution (as in 2-monoalkyl-substituted or 2,2-dihydro analogues) greatly impacted the transcriptional competency of the AR−ligand complex. It was more specifically observed that tetrahydro analogue **8** (LG121071) exhibited tighter binding affinity than the substituted analogues and scored as a full agonist in cotransfection assays, which led us to investigate the ability of **8** to exhibit AR agonist activity in a classic animal model.

Chemistry. Our previously reported efforts at construction of 2,2-dialkyl-substituted pyridonoquinolines involved sequential annulation of each terminal ring about a central core through cyclization strategies.[13] Synthesis of 1,2,3,4-tetrahydro-8-pyridono[5,6-*g*]quinolines was achieved in a more efficient manner from a single annulation onto an existing quinoline core after appropriate functionalization (Scheme 1).[14] The requisite intermediate 4-ethyl-1,2,3,4-tetrahydroquinoline (**6**)[15] was synthesized in a two-step sequence starting from commercially available lepidine (**4**). Treatment of **4** with LDA[16] and trapping of the resultant anion with iodomethane furnished 4-ethylquinoline (**5**)[17] in excellent yield (98%). Reduction of the quinoline ring using $NaBH_4-NiCl_2$[18] afforded the required 4-ethyltetrahydroquinoline **6** in 96% yield. Standard nitration and subsequent reduction by catalytic hydrogenation provided diamine **7**, which smoothly underwent Knorr cyclization[19] (ethyl 4,4,4-trifluoroacetoacetate, $ZnCl_2$,

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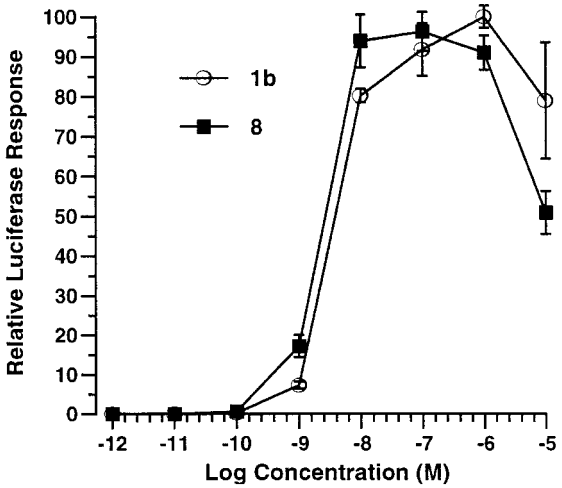

Figure 1. hAR agonist dose response of compound **8** in cotransfected CV-1 cells. Values represent mean ± SEM of at least triplicate determinations.

Table 1. hAR Agonist and Antagonist Activity in Cotransfected CV-1 Cells and Binding Affinities for hAR in Transiently Transfected COS-1 Cells[a]

	hAR agonist activity		hAR antagonist activity		hAR binding
compd	EC_{50}[b] (nM)	efficacy[c] (%)	IC_{50}[b] (nM)	efficacy[c] (%)	K_i[b] (nM)
1b	5 ± 1	100 ± 0	na[d]		3 ± 1
2	0.3 ± 0.1	128 ± 12	na		4 ± 1
8	4 ± 1	100 ± 7	7481 ± 1655	36 ± 10	17 ± 3

[a] Cotransfection assay experiment values represent at least triplicate determinations. [b] Values represent mean ± SEM. EC_{50} values represent the concentration of ligand required to give half-maximal activation; IC_{50} values represent the concentration of ligand required to give half-maximal inhibition of DHT at its EC_{50}. [c] Efficacies were compared to that of dihydrotestosterone (100%). [d] Not active; defined as efficacy < 20%, potency > 10 000 nM.

EtOH, reflux) to afford 4-ethyl-1,2,3,4-tetrahydro-6-(trifluoromethyl)-8-pyridono[5,6-*g*]quinoline (**8**).

In Vitro and in Vivo Biological Activity. 1. Cotransfection and Binding Assays. The AR agonist activities of **8** as well as that of the known AR agonists **1b** and **2** were studied experimentally in a cellular background through both ligand-dependent stimulation of reporter gene (luciferase) induction using the cotransfection assay[20] (Figure 1) and a whole-cell receptor binding assay (Table 1). Also included in Table 1 are data for the compound and standards tested in cotransfection assays using hAR in the antagonist mode in the presence of DHT at its EC_{50}. Activities on other IRs including human progesterone receptor (hPR-B), human glucocorticoid receptor (hGR), human mineralocorticoid receptor (hMR), and human estrogen receptor (hER) were also determined, and there was found to be no agonist or antagonist response induced by compound **8**.[21]

2. Two-Week LH Suppression Assay in Rats. The hypothalamic−pituitary axis in male rats and men functions as a feedback loop to regulate circulating levels of endogenous steroid (T, DHT) and gonadotropins [luteinizing hormone (LH), follicle-stimulating hormone (FSH)]. Castration causes a dramatic increase in secretion of pituitary LH, and restoration of androgen control and subsequently restoration of LH to normal physiologic levels can be achieved by administration of exogenous androgens.[22] Compound **8** was examined in this established model of androgen action to assess its ability to suppress castration-induced elevation of serum LH after oral administration (Figure 2).

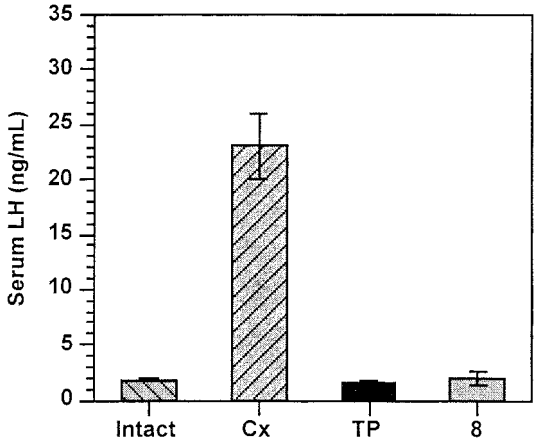

Figure 2. Suppression of luteinizing hormone (LH) in castrated (Cx) mature rats by compound **8** (20 mg/kg, po) or testosterone propionate (TP) (1 mg/kg, sc) daily for 2 weeks ($n = 4$ for all groups). All values represent the mean serum LH concentrations ± SEM.

Results and Discussion. Compound **8** stimulates reporter gene expression in a concentration-dependent manner in the cotransfection assay, with a potency and efficacy equivalent to that of DHT (Figure 1). A slight antagonist response is observed only at the highest concentration (10 μM) in the cellular background used for this assay. In vivo, testosterone propionate administered subcutaneously at a dose of 1 mg/kg completely blocked the effects of castration, restoring serum LH levels to that of the intact control animals. Compound **8** at a dose of 20 mg/kg administered orally was also fully efficacious at suppressing the castration-induced elevation of LH in the male rat.

Conclusion. The data shown for LG121071 (**8**) represent the discovery of the first known orally active, nonsteroidal AR agonist. This finding, together with earlier reports from these laboratories,[23] provides further support for a drug discovery approach targeting both agonists and antagonists of sex steroid hormone receptors diverging from a common pharmacophore. Compounds based on this novel template are the subjects of continued investigations toward development of therapeutically useful AR agonists with desirable tissue selectivity and avoiding structure-based side effects associated with compounds derived from steroidal templates.

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Supporting Information Available: Synthetic procedures and chemical characterization data for compounds **5**−**8** and biological assay methods. This material is available free of charge via the Internet at http://pubs.acs.org.

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